Aggregated oviposition in *Actinote pellenea pellenea* Hübner (Lepidoptera: Nymphalidae)

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Abstract. The oviposition pattern of *Actinote pellenea pellenea* on *Austroeupatorium inulaefolium* was investigated in two coastal sites in SE Brazil to test if there is any pattern of preference for host plant traits. At both sites, host plants were marked and measured for height, distance of the nearest plant, number of leaves with *A. p. pellenea* immatures (eggs and first instar larvae), number of groups of immatures, and total number of eggs per plant. An apparency index was calculated relating plant size to distance from its three nearest conspecific neighbours. Total leaf area, orientation and inclination were recorded for each leaf with a group of immatures. There was no significant correlation between the number of ovipositions on each plant and habitat and plant characteristics as plant height and apparency index. The number and density of eggs by oviposition was not correlated with leaf characters as area, orientation or inclination. At both sites clusters of immatures showed a grouped distribution, with some plants having more immatures than predicted by chance. In the only case of a double oviposition on the same leaf, the later cluster was significantly smaller. The results show that distribution of eggs - reflecting female choice - was not related with the above measured plant and leaf traits. However, the results do suggest that females probably choose plants where immatures are already present, resulting in the observed grouping pattern.

Key words: *Actinote*, *Austroeupatorium*, host plant selection, oviposition.

Introduction

For most holometabolous insects in general, and with herbivores in particular, adults are more mobile than immatures, with the decisions of ovipositing females often critical for the fitness of the offspring (Doak *et al.*, 2006). For most herbivorous insects, host plant acceptability and quality vary strongly among plant species, populations and individuals and even among different plant parts (Strong *et al.*, 1984; Price, 1997; Kerpel *et al.*, 2006). The ability of females to choose among different hosts or host parts has important consequences on their offspring's performance, with the females usually using plant cues to identify the most suitable food resource. Many different cues are used by females during the oviposition process. These include secondary compounds, visual signals (plant and leaf shape), presence of natural enemies or mutualists, presence of conspecific immatures, microclimate (Rausher, 1978; Williams & Gilbert, 1981; Freitas & Oliveira, 1996)

and plant vigour (Price, 1991, 1997). Furthermore, ovipositing females can also respond to indirect cues such as leaf age and size, internode length (Price *et al.*, 1987; Freitas *et al.*, 1999), plant apparency, grouping and position of host plants across the landscape (Feeny, 1976; Courtney & Courtney, 1982; Mackay & Singer, 1982).

The Neotropical genus *Actinote* Hübner, [1819] (Nymphalidae: Heliconiinae: Acraeini) has 31 described species distributed through Central and South America, reaching maximum diversity in the montane regions in the Andes and Southern Brazil (Francini *et al.*, 2004; Lamas, 2004; Paluch, 2006; Paluch *et al.*, 2006; Silva-Brandão *et al.*, 2008). All known species feed on Asteraceae and are gregarious during all stages (Francini, 1989, 1992; Paluch *et al.*, 2005; Freitas *et al.*, 2009).

The widespread *Actinote pellenea* Hübner, [1821] has 17 recognized subspecies distributed across all South America from Colombia to Northern Argentina found over a wide variety of secondary and open habitats (Francini, 1989, 1992; Paluch, 2006). In coastal Southern Brazil, *A. pellenea pellenea* Hübner, [1821] is very common with four or five generations per year. Here larvae of *A. p. pellenea* feed on three species of Asteraceae: the vines *Mikania micrantha* and *Mikania cordifolia*, and the shrub *Austroeupatorium*

inulaefolium (Francini, 1989, 1992) (Fig. 1).

The following study describes the pattern of oviposition distribution of *A. p. pellenea* on patches of *A. inulaefolium*, and discusses the factors that may explain the observed patterns.

Methods

Study sites

The study was carried out at two coastal sites of São Paulo State, Southern Brazil: 1) Xixová-Japuí State Park (JAPUI), São Vicente, São Paulo (23°59' S, 46°23' W), in July 1st and 2nd , 1991, and 2) the valley of the Cubatão river, (VRCUB), Cubatão, São Paulo (23°53' S, 46°27' W) during November 12, 1992. Both sites are covered by lowland subtropical forest (Ururahy *et al.*, 1984). Annual rainfall reaches 2500 mm and the average annual temperature is 21°C (Setzer, 1949; Nimer, 1989). Field work was conducted along open trail edges and in early succession stages secondary vegetation.

Sampling procedures

At each site all individuals of *A. inulaefolium* present along a previously defined linear transect of 200 m were sampled, including all plants to a 5 meters distance on both sides of the transect, including all nearby plants in the area. Immatures of *A. p. pellenea* of each host plant were recorded and all ovipositions were collected for laboratory work. Each individual plant was tagged and recorded for height (five classes of 100 cm), distance of the nearest plant (in meters), orientation in relation to the nearest plant (in degrees), number of leaves with immatures, total number of immature groups and total number of immatures. For each leaf with immatures, the area, inclination (to the horizontal), orientation (in degrees to the central axis of the plant), amount of herbivory and number of immatures per group were recorded. An "apparency index" (AI, following Feeny, 1976) was calculated for each individual plant, as: AI = [(Ho - Ha + DA) + (Ho - Hb + DB) + (Ho - Hc + DC)] / 3; where Ho = plant height, Ha, Hb, Hc = height of the nearest three plants of the species, and DA, DB, DC = distance of the nearest three plants of the same species present along the linear transect. This index is lowest (including negative values) when the distance between a pair of plants is less than the lower plant of the pair. The index increases with increasing distance between the two plants. To determine the distribution of the plants, the study area was included in a 108 m² rectangle, and this was subdivided in 27 squared

sectors of 2x2 m (three rows and nine columns). The distribution pattern of plants was determined using the index of dispersion (Ludwig & Reynolds, 1988) with values < 1 indicating a uniform distribution, = 1 a random distribution and > 1 a grouped distribution.

Inclination and orientation of each leaf was estimated with compass and protractor with a precision of 1°. Each oviposition was assigned to one of three developmental stages based on predominant egg colour following Francini (1989), where Y = yellow (0-1 day after oviposition), O = orange (1-3 days after oviposition), R = red (more than 3 days after oviposition), B = black (1 or two days before eclosion). First instars were also considered in the present study, since larvae usually remain, together with their empty egg shells, on the same leaf for the first days following eclosion.

All measured leaves were scored into four categories of herbivore damage: (0) 0%; (1) up to 10%; (2) 11% to 25%; (3) 26% to 50%; (4) more than 51%. Since the leaves of *A. inulaefolium* are nearly rhombus-shaped, leaf area (in cm²) was estimated using the formula (L*W)/2, where L = length and W = width.

Egg density for each oviposition was estimated from the average of five independent counts of eggs over different sections of each oviposition event, giving the number of eggs per cm². The number of eggs in each oviposition was estimated as the product of the average density by the area of the oviposition.

Results

Host plant density. At JAPUI 23 plants of *A. inulaefolium* were inspected showing a density of 0.21 plants/m² with a non-random distribution (Poisson test, $\chi^2 = 4.2031$, DF = 2, p < 0.05). The dispersion index was 1.331, indicating that the plants are grouped. At VRCUB 11 plants were inspected with a density of 0.10 plants/m² with a random distribution (Poisson test; $\chi^2 = 0.7365$, DF = 1, p > 0.70) and dispersion index of 0.8042, which indicated a homogenous distribution.

Host plant height. At JAPUI *A. inulaefolium* height ranged from 100 to 500 cm ($\bar{x} = 213.0$ cm, SD = 109.98, n = 13), significantly lower than from VRCUB, that ranged from 300 to 700 cm ($\bar{x} = 472.7$ cm, SD = 110.37, n = 11) (t test = -6.4339, DF = 32, p < 0.001). At JAPUI the AI (apparency index) ranged from -100 to 1760 while at VRCUB the range was greater, from 800 to 3040, indicating that plants with high apparency co-occur with plants with low apparency. There was no correlation between the AI and plant height at either site (Spearman, r = 0.0689 in JAPUI, t = 0.3163, DF = 21, p > 0.75, and r = 0.1908 in VRCUB, t = 0.5831, DF

= 9, P > 0.57).

Distribution of immatures. At JAPUI 43 groups of immatures (eggs or first instar larvae) were recorded on 42 leaves of all plants with the number of groups per plant ranging from 0 to 9 ($\bar{x}$ = 1.9 ovipositions/plant, SD = 2.40, n = 23) and a dispersion index of 3.078. At VRCUB 41 groups of immatures were recorded on 41 leaves of all plants, with a range from 0 and to 11 ($\bar{x}$ = 3.7 ovipositions/plant, SD = 12.82, n = 23) and a dispersion index of 3.439. The values of the dispersion indexes indicate that distribution of immatures on the plants of both sites was grouped. Ovipositions were recorded on leaves ranging from 15 to 450 cm above ground at JAPUI and from 130 to 500 cm at VRCUB. When grouped into intervals of 100 cm, the data showed a concentration of 27 ovipositions within the interval 101 - 200 cm in JAPUI, and of 16 ovipositions in the interval 201 - 300 cm in VRCUB. A double oviposition on the same leaf was observed once during the study.

Oviposited leaves. The average surface area of leaves that showed oviposition at JAPUI ($\bar{x}$ = 2553.21 mm2, SD =1929.36, n = 58) was significantly lower than at VRCUB ($\bar{x}$ = 4549.3 mm2, SD = 2653.82, n = 25) (t test = -3.8755, p < 0.0002, DF = 81). There was no preferential orientation of oviposited leaves at either site (JAPUI χ^2 = 2.00, p = 0.57, DF = 3; VRCUB χ^2 = 3.37, p = 0.34, DF = 3). With respect to leaf inclination, most oviposited leaves were between 10° and 20° from the horizon at both sites. Considering only leaves showing oviposition, most were leaves with low herbivory damage (0 to 10% herbivory) at both JAPUI (67.6%) and VRCUB (92.6%). However, because the patterns of leaf orientation and inclination, and herbivory, were not evaluated for all plants, these data are not useful in showing tendencies or preferences by ovipositing females.

Eggs x plants. At JAPUI the number of eggs per plant ranged from 0 to 3850, with a total of 20,100 eggs from 14 of 23 plants ($\bar{x}$ = 873.9 eggs/plant, SD = 1154.42) and with a dispersion index of 1525, a significant grouped pattern. No significant correlation was determined among the following parameters: number of eggs and plant height (Spearman, r = 0.1179, t = 0.5442, DF = 21, p > 0.59), number of ovipositions and plant height (Spearman, r = 0.0982, t = 0.4521, DF = 21, p > 0.66), the AI and number of eggs per plant (Spearman, r = -0.1485, t = -0.6879, DF = 21, p > 0.49), or the AI and number of ovipositions per plant (Spearman, r = -0.3571; t= -1.7521, DF = 21, p < 0.09). At VRCUB the number of eggs per plant ranged from 338 to 4236 (all plants with at least one oviposition) giving a total of 15,880 eggs on 11 plants ($\bar{x}$ = 1443.6 eggs/plant, SD = 1399.91) and with a

Figure 1. A female *Actinote pellenea pellenea* ovipositing in *Austroeupatorium inulaefolium*.

dispersion index equal to 1357.55. These data also revealed a significant grouped pattern. Again, as at JAPUI, no significant correlation was demonstrated between the following parameters: number of eggs and plant height (Spearman, r = 0.1908, t = 0.5830, DF = 9, p > 0.57), number of ovipositions and plant height (Spearman, r = 0.2435, t = 0.5392, DF = 9, p > 0.6), the AI and number of eggs per plant (Spearman, r = 0.3184, t = 0.31, DF = 9, p > 0.76), or the AI and the number of ovipositions per plant (Spearman, r = 0.3184, t = 1.0078, DF = 9, p > 0.33).

Eggs x leaves. At JAPUI the average density of eggs ranged from 111 to 470 eggs/cm² ($\bar{x}$ = 259.5 eggs/cm², SD = 68.32, n = 57) with the number of eggs per oviposition ranging from 86 to 1266 ($\bar{x}$ = 479.2 eggs, SD = 228.93, n = 56). There was no significant correlation among the following parameters: number of eggs and leaf area (Spearman, r = 0.1054, t = 0.7934, DF = 56, p < 0.43), number of eggs and leaf orientation (Spearman, r = -0.0114, t = -0.0856, DF = 56, p > 0.93), or number of eggs and leaf inclination (Spearman, r = 0.0144, t = 0.1078, DF = 56, p > 0.91). At VRCUB the average density of eggs was from 95 to 374 eggs/cm² ($\bar{x}$ = 238.5 eggs/cm²; SD = 66.81, n = 27) with the number of eggs per oviposition ranging from 100 to 883 ($\bar{x}$ = 422.31 eggs, SD = 199.01 eggs; n = 26). And again there was no significant correlation among the parameters: number of eggs and leaf area (Spearman, r = -0.2736, t = -1.3644, DF = 23, p > 0.18), number of eggs and leaf orientation (Spearman, r = -0.0415, t = -0.1991, DF = 23, p > 0.84), or number of eggs and leaf inclination (Spearman, r = 0.0327, t = 1.1571, DF = 23, p < 0.87). Correlations between leaf area and

egg density were also not significant at either JAPUI (Spearman, r = -0.718, t = -0.339, DF = 55, p > 0.9) and in VRCUB (Spearman, r = -0.1327, t = -0.6419, DF = 23, p > 0.52).

Discussion

Our study was not conclusive in revealing any consistent pattern of oviposition in *A. p. pellenea*, except for clearly indicating that ovipositions tend to be grouped. The morphological plant traits investigated by our study were apparently not used by the females when selecting oviposition sites. There are of course several additional factors that would be important in selection by oviposition sites by *A. p. pellenea* females that were not evaluated in our study, such as: 1) other plant features, such as secondary compounds, nutritional quality and/or vigor (Kerpel *et al.*, 2006), 2) presence of alternative host plants in the same area (the common scandent vines *M. micrantha* and *M. cordifolia*) that might influence the patterns we found, and 3) a strong preference for plants previously oviposited by females (Ulmer *et al.*, 2003). Despite of which factors are influencing female choice, it is worth noting that the grouped pattern of immatures was revealed at both sites. As a result, many plants were not used for oviposition females at all, by contrast to a few that received up to nine ovipositions (> 3000 eggs). The advantages of grouped eggs are well known for many species of Lepidoptera, including protection against desiccation and predation (Stamp, 1980; Clark & Faeth, 1998). Gregarious larvae from egg clusters also benefit from increased development rates and survival as well as reduced predation and parasitism (Lawrence, 1990; Clark & Faeth, 1998; Denno & Benrey, 1997). On the other hand, as pointed out above, super-oviposition results in high mortality of small larvae which will not get enough food as we frequently observed in the field. The reasons for this grouped pattern of immatures in *A. p. pellenea* require further investigation to reveal if the advantages in many larvae feeding in the same plant are higher than the risk of death by starvation. Additionally comparisons with other species of *Actinote* are also needed to reveal if the pattern of group immatures can be generalized in this genus.

Acknowledgements

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